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Seasonally dependent testicular apoptosis in the tropical Long-fingered bat (*Miniopterus inflatus*)

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Abstract

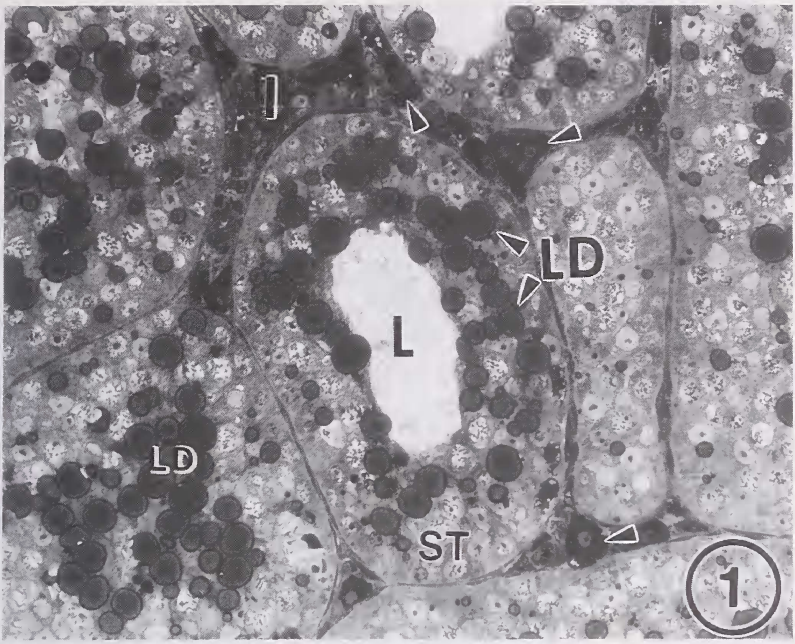
Testicular morphology of long-fingered bats trapped in March and early April (a period of sexual dormancy) was studied using both light and electron microscopy. The interstitial tissue, generally smaller in proportion to the seminiferous tubules, was largely made up of compactly arranged interstitial (Leydig) cells. Physiological cell death (apoptosis) was characterized by the occurrence of dense cytoplasm obscuring most of the subcellular organelles, myelin-like whorls of residual bodies and lipid inclusions in the cytoplasm, and roughly spheroidal nuclei. Normal mitochondria were round in outline. Some of these apoptotic cells were phagocytosed by the interstitial tissue macrophages. In the seminiferous tubules this degenerative process was marked by spermatogonial karyolysis, apoptosis of spermatocytes and extensive accumulation of large lipid droplets in the Sertoli cell cytoplasm. The tubular walls and lumen were completely devoid of spermatids and spermatozoa.

These observations suggest that the sexual dormancy in these bats is characterized by a marked apoptosis of testicular micro-structural components hitherto unreported.

Introduction

Long-fingered bats are members of the Miniopterinae subfamily occupying varying ecological niches ranging through most of the tropical old world; Africa, Madagascar, southern Europe, South-East Asia to Philippines, New Caledonia, Loyalty Islands and the northern areas of Australia (Kingdon 1974; Walker 1975; Hill and Smith 1984). Within the subfamily, there are eleven species (Hill and Smith 1984) of which three are well-known and widely distributed in Africa. These are; *M. minor* whose distribution is restricted to the tropical coast of Africa, Congo river and islands off Africa; *M. schreibersi* found in Australia, South-East Asia and southern Europe stretching down to Africa and the cape; and *M. inflatus* which is largely distributed in tropical Africa and sometimes found in areas adjacent to the more widely distributed *M. schreibersi* (Kingdon 1974; Smithers 1983). The distinction among the three species is often less clear-cut and measurements particularly those of the skull have been used to differentiate the individual members. The bats live in groups or clusters and primarily inhabit caves where they roost in dark crevices. They feed mainly on flying insects.

Reproduction in the temperate long-fingered bats is a unique phenomenon whereby mating occurs prior to winter hibernation and the reproductive activity remains dormant throughout the hibernation period (Courrier 1927; Gustafson 1979). Thus in females, the ovum is fertilized before hibernation and further development of the embryo is minimal during the same period (Kingdon 1974). In males, however, the reproductive system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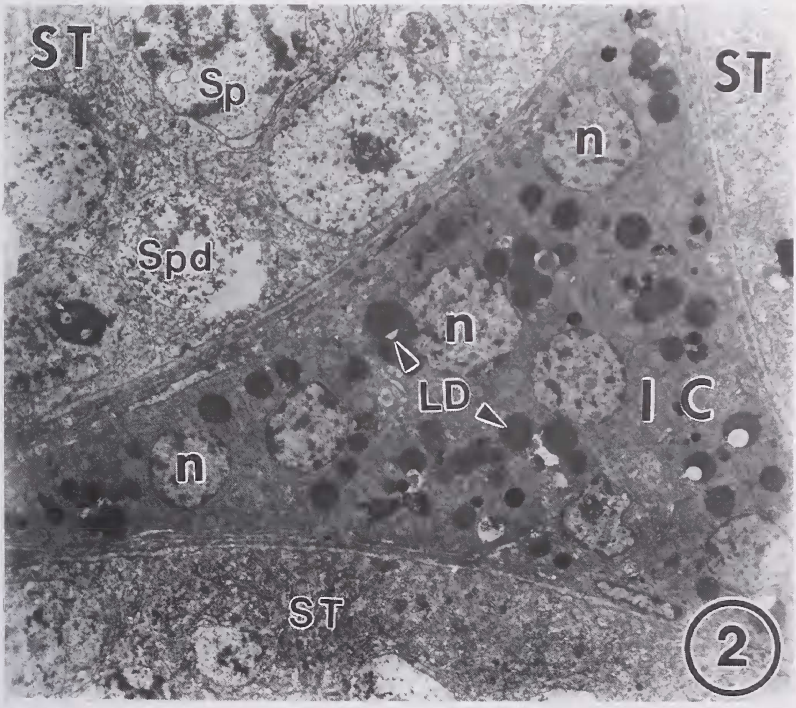
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regresses after mating but prior to hibernation (Courrier 1927). Available reports on the tropical species, *M. minor*, of the coastal region of Kenya similarly suggest a period of testicular degeneration followed by recrudescence (McWilliam 1988). These reports appear to have been based on behavioural as well as macro-anatomical observations. Consistently missing, however, are the micro-structural accounts of these degenerative changes. This study therefore aims at investigating the testicular micro-anatomical changes associated with the degenerative process in these bats during dormancy.

Material and methods

Thirteen bats trapped between March and early April at Mt. Suswa a few kilometers from Naivasha, Kenya were immediately transported to the laboratory and kept in temporary roosts overnight before commencement of the study. At the time of study, the bats were anaesthetized using chloroform, perfused with phosphate buffered 2.5% glutaraldehyde through cardiac puncture and the perfusate drained via the caudal vena cava. The testes were dissected out of the scrotum and further immersed in the same fixative for 24 hours. The fixed testes were then removed from the fixative, trimmed of excess fatty tissue, diced into approximately 1 mm cubes and processed for light and electron microscopy. In brief, the cubes were washed in phosphate buffer, post-fixed in osmium tetroxide, dehydrated in ascending concentrations of ethyl alcohol and embedded in epoxy resin mixture. Approximately 1 mm thick sections were obtained from the embedded tissues, stained with toluidine blue and observed under the light microscope. From the same blocks, 60 nm thick sections were obtained, mounted on 300 mesh copper grids, stained with uranyl acetate and counter-stained with lead citrate. The stained sections were then viewed under the Zeiss 10 electron microscope.

Results

Histological observations on the testes showed that the seminiferous tubules comprised a large volume of the parenchyma compared to the interstitial tissue which were generally restricted to the narrow intertubular spaces (Fig. 1). The interstitial tissue consisted of a continuous sheet of compactly arranged interstitial cells of Leydig held together by connective tissue fibres. The nuclei of the interstitial cells were roughly spheroidal and predominantly euchromatic. The cytoplasm was dense, abundant and contained accumulations of lipid droplets and roughly spheroidal mitochondria while the rest of the subcellular organelles were poorly discerned (Figs. 2, 3). In some cells, myelin-like whorls of residual bodies were encountered (Fig. 3). Interstitial tissue macrophages appeared to be phagocytizing the degenerating interstitial cells (Fig. 4). The walls and lumen of the seminiferous tubules were devoid of spermatids and spermatozoa respectively (see Fig. 1). The tubular epithelium appeared to be undergoing various degrees of degenerative changes. Spermatogenic cell changes appeared as either occasional spermatogonial karyolysis (Fig. 5) or apoptosis of the primary spermatocytes (Figs. 2, 6) among apparently normal cell popula-

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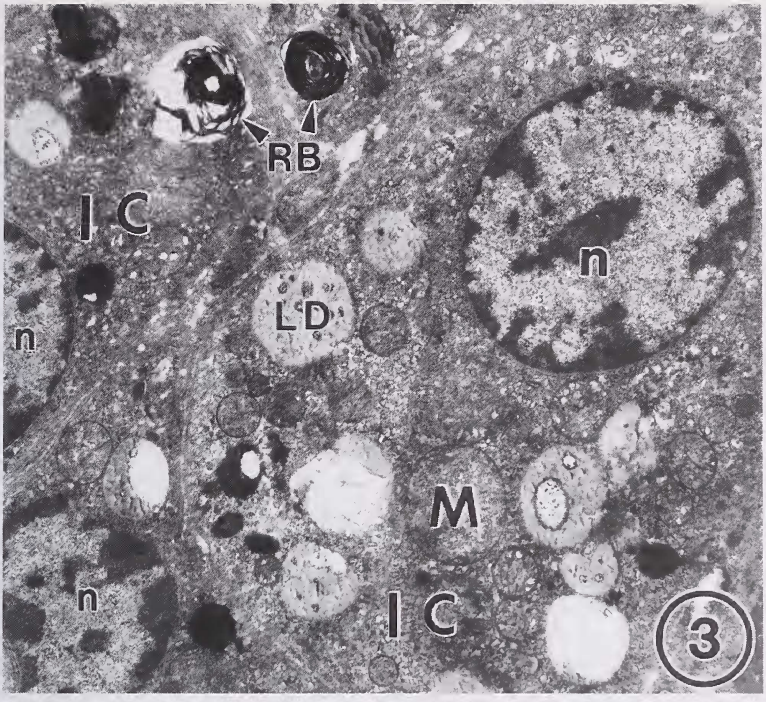
Fig. 1. A photomicrograph of a semithin (1 mm thick) section of plastic embedded testis of the long-fingered bat (*Miniopterus inflatus*). The interstitial tissue (I), mainly comprising compactly arranged interstitial (Leydig) cells, occupy narrow intertubular spaces. The cytoplasm of these interstitial cells have aggregations of lipid droplets (arrow heads). Seminiferous tubules (ST) show conspicuous accumulation of numerous and unusually large lipid droplets (LD) mainly distributed in the apical cytoplasm. The tubular wall and lumen (L) are devoid of spermatids and spermatozoa respectively. Mag. ×400.

Fig. 2. Electron micrograph of the interstitial tissue and parts of seminiferous tubules (ST). The interstitial cells (IC) show spheroidal nuclei (n) and dense cytoplasm with lipid droplets (LD). Note that most of the subcellular organelles are obscured by the dense cytoplasm. Degenerating spermatogenic cells (Spd) occur in the tubules among the normal spermatocytes (Sp). Mag. ×2,500.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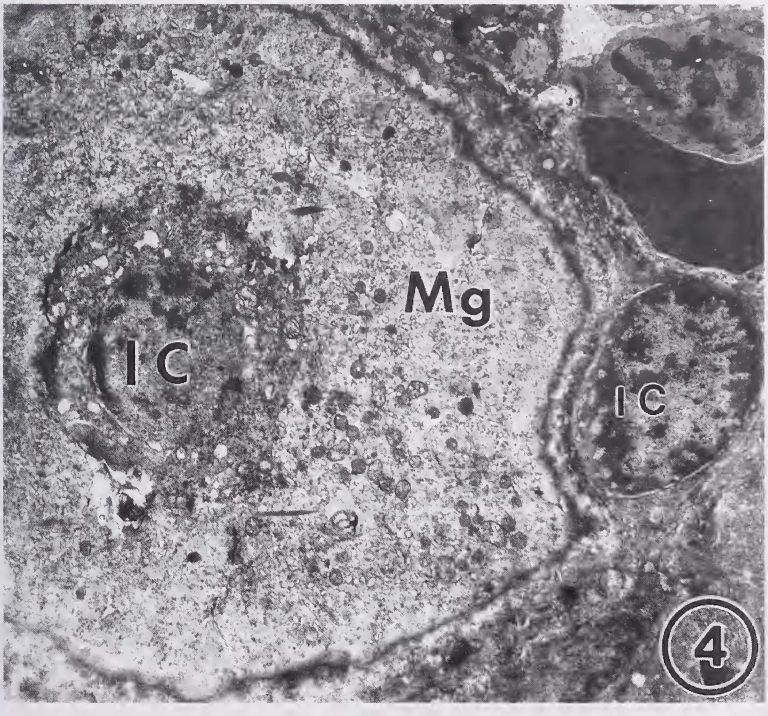
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tions. As a result of the disintegration of spermatogenic cell membrane during apoptosis, the cellular organelles (especially the smooth endoplasmic reticulum) were scattered within the Sertoli cell cytoplasm (Fig. 7). Numerous and unusually large lipid droplets were distributed in the apical cytoplasm of the Sertoli cells (Figs. 1, 8).

Discussion

Bats exhibit varied breeding patterns with some, mainly the hibernating Rhinolopidae and Vespertilionidae showing an asynchronous seasonal reactivation of the primary and secondary sexual organs (Courrier 1927; Kingdon 1974; Gustafson 1979; Racey 1982; Merwe and Rautenbach 1989; Happold and Happold 1990; Krutzsch and Crichton 1990). Non-hibernating bats, on the other hand, breed all the year round (Kingdon 1974; Krutzsch 1979). The long-fingered bat (a Vespertilionid), however, shows an extreme kind of seasonal breeding. Behavioural and macro-anatomical studies have shown that the females experience delayed implantation corresponding to the time when males undergo sexual dormancy (Gustafson 1979; Racey 1982) characterized by degenerative changes in the reproductive organs (Courrier 1927; McWilliam 1988). The present microscopical observations in the dormant males confirm the previously reported behavioural and macro-structural changes.

The volume of seminiferous tubules compared to that of the interstitial tissue can be used to group various animal species (Fawcett et al. 1973). In this study the long-fingered bats resemble rats, mice and guinea pigs in having a large volume of seminiferous tubules among which are small quantities of interstitial tissue. The occurrence of poorly defined cytoplasmic organelles, myelin-like whorls of residual bodies and accumulation of lipid droplets in the apparently dense and abundant cytoplasm of the interstitial (Leydig) cells are suggestive of a period of dormancy accompanied by physiological cell death otherwise referred to as apoptosis (Davies 1984). In normal circumstances, active interstitial cells secreting testosterone are characterized by the presence of smooth endoplasmic reticulum, mitochondria, Golgi apparatus and lipid inclusions in the cytoplasm (Christensen and Gillim 1969; Christensen 1975). As expected therefore, a depression in interstitial cell activity inevitably leads to a reduction in the volume of these organelles (Sinha Hikim et al. 1993). Although these investigators reported no effect on testosterone levels during early regression, previous studies in the cape horse-shoe bat (Bernard 1986) and Schreiber's long-fingered bat (Bernard et al. 1991) during the period of dormancy showed a marked reduction in testosterone production related to the status of subcellular organelles. Hence, the occurrence of dense cytoplasm with poorly defined organelles may suggest a phase of reduced steroidogenic activity and testosterone production. Accumulation of lipid inclusions in the cytoplasm of inactive cells is not an uncommon finding. In the ovary of the leaf-nosed bat (*Macrotus californicus*), for example, the accumulation of lipid droplets and reduction of smooth endoplasmic reticulum content was associated with a depression in steroid secretion (Crichton et al. 1990). Similarly such accumulation has been associated with cell inactivity in the testes of a non-breeding fossorial tropical rodent, the naked mole-rat (Onyango et al. 1993). The myelin-like whorls of residual bodies

Fig. 3. An electron micrograph of the interstitial cells (IC) showing features of degeneration namely; dense cytoplasm containing poorly outlined subcellular organelles, accumulation of lipid droplets (LD) and myelin-like whorls of residual bodies (RB), and presence of spheroidal and euchromatic nuclei (n). Normal spheroidal mitochondria (M) also occur. Mag. ×8,000.

Fig. 4. An electron micrograph showing a testicular macrophage (Mg) phagocytizing degenerative interstitial cells (IC). Mag. ×5,000.

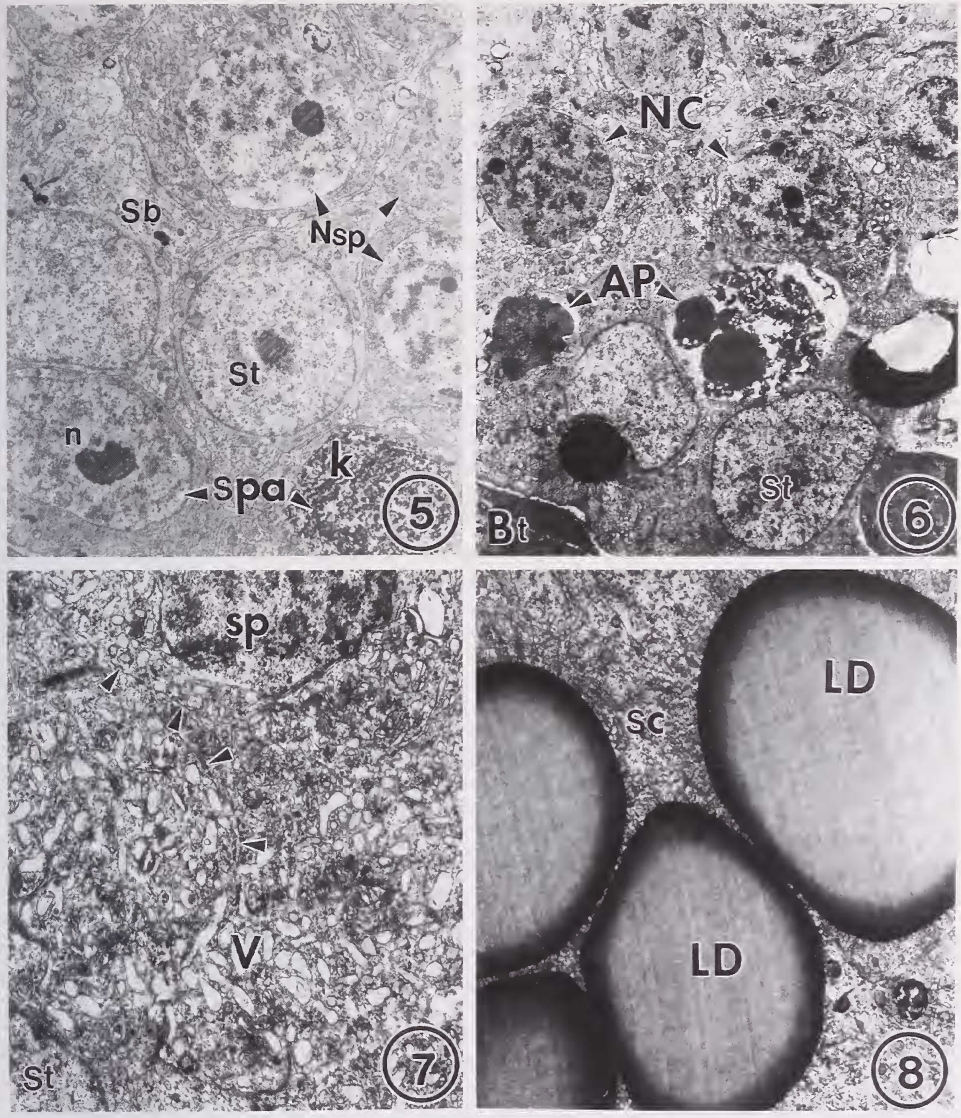

Fig. 5. A photomicrograph of a part of the seminiferous tubule showing two spermatogonia (Spa); one undergoing karyolysis (k) and a normal one (n). Normal spermatocytes (Nsp) are surrounded by Sertoli cell branches (Sb). St = Sertoli cell. Mag. ×3,200.

Fig. 6. A photomicrograph of a part of the seminiferous tubule showing some apoptotic primary spermatocytes (AP) and neighbouring normal spermatocytes (NC). St = Sertoli cell; Bt = Boundary tissue. Mag. ×2,520.

Fig. 7. An electron micrograph showing presence of spermatogenic cell vesicles (V) in the Sertoli cell cytoplasm (St)-an indication of phagocytosis. A poorly defined spermatogenic cell nucleus (Sp) and remnants of the disintegrating cell membrane (arrow heads) are apparent. Mag. ×6,480.

Fig. 8. An electron micrograph of the apical cytoplasm of a Sertoli cell (Sc) with accumulation of very large lipid droplets (LD). Mag. ×4,000.

are thought to originate from the mitochondria (Sandborn 1970; Davies 1984). There are speculations that they may represent the degenerative form of mitochondria (Davies 1984). If this is true then their existence in these cells at the time of study serves to emphasize the thesis that these cells were indeed degenerating. Furthermore, there is evidence here suggesting that these degenerating cells were being phagocytosed by the testicular macrophages.

The degenerative process in the seminiferous tubule is marked by the occurrence of karyolytic spermatogonia and apoptotic spermatocytes in the tubule, sequestered cytoplasmic materials of spermatogenic cells and accumulation of unusually large amounts of lipid droplets in the Sertoli cell cytoplasm. It is probably due to the observed karyolysis and apoptosis of the immature spermatogenic cells that the spermatids and spermatozoa were absent altogether from the tubular wall and lumen. One of the primary functions of the Sertoli cells is to phagocytose the degenerating spermatogenic cells (Soderstrom and Nikkanen 1979; Fawcett 1986). In connection with this, the observation of the spermatogenic cell organelles in Sertoli cell cytoplasm in this case may imply phagocytosis. The large accumulation of apically disposed lipid droplets in the Sertoli cell cytoplasm is rather unusual though similar findings have previously been reported. In hypospermatogenic men large quantities of lipid droplets were also encountered in the Sertoli cell cytoplasm (Soderstrom and Nikkanen 1979). In *Myotis lucifugus lucifugus* (Gustafson 1987), seasonal changes in lipid inclusions were reported in the seminiferous tubules though no specific reference was made to Sertoli cells. Ordinarily, Sertoli cells participate to some degree in androgen biosynthesis (Christensen 1965; Pudney 1986). Therefore the accumulation of these inclusions in the Sertoli cells of these bats may indicate an impaired utilization of cholesterol reservoirs, hence, a reduction in androgen biosynthesis. The present study has not addressed the issue of regeneration time of various testicular cell populations during recrudescence. We hope to follow this up in our next study.

In conclusion, the micro-structural apoptosis of testicular tissue observed in this study generally confirms the previously described behavioural and macro-structural changes in the long-fingered bats during dormancy (see Courrier 1927; McWilliam 1988). However, of particular interest are the causes of these degenerative changes. Although this is beyond the scope of this work, it is speculated that the intricate paracrine inter-relationship among the various testicular cells may play a part. It is now widely believed that there is a complex inter-relationship between the testicular interstitial cells of Leydig and Sertoli cells, Sertoli and spermatogenic cells, and among the spermatogenic cells themselves (Krester et al. 1991; Sharpe et al. 1990, 1993). This bat species therefore provides an excellent model for studying these influences.

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Zusammenfassung

Saisonabhängige Apoptose im Hoden der tropischen Langflügelfledermaus (Miniopterus inflatus)

Hoden von Langflügelfledermäusen der Art *Miniopterus inflatus* wurden während einer sexuellen Ruhephase in den Monaten März und April licht- und elektronenmikroskopisch untersucht. Das interstitielle Gewebe, im allgemeinen relativ klein proportional zu den Tubuli contorti seminiferi bestand überwiegend aus kompakt arrangierten interstitiellen (Leydig) Zellen. Physiologischer Zelltod (Apo-

ptose) wurde gekennzeichnet durch das Vorkommen von dichtem Cytoplasma bei verborgenen Zellorganellen, myelinähnlich gewundenen Residualkörpern und Lipideinschlüssen im Cytoplasma und annähernd kugelförmigen Zellkernen. Die normalen Mitochondrien erschienen im Umriß abgerundet. Einige dieser apoptotischen Zellen wurden von im interstitiellen Gewebe auftretenden Makrophagen phagozytiert. In den Tubuli contorti seminiferi waren diese degenerativen Prozesse markiert durch Spermatogonienkaryolyse, Apoptose von Spermatocyten und starke Ansammlung von großen Lipidtropfen im Cytoplasma der Sertoli-Zellen. In den tubulären Wandungen und den Hohlräumen befanden sich weder Spermatiden noch Spermien. Diese Beobachtungen lassen annehmen, daß der sexuelle Ruhezustand der männlichen Fledermäuse durch ausgeprägte Apoptose mikrostruktureller Komponenten bedingt ist.

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